

WHAT IS CLAIMED IS:

1. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises
 - (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line, at least one kinase, and at least one arrestin in a suitable buffer;
 - (b) separating the GPCR bound arrestin from the unbound arrestin; and
 - (c) determining the level of GPCR bound arrestin,whereby a compound which is an agonist of said GPCR is identified when the level of GPCR bound arrestin is raised relative to a control.
2. A method according to claim 1, wherein said kinase is a G-protein coupled receptor kinase (GRK).
3. A method according to claim 1, wherein said arrestin is β -arrestin.
4. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises
 - (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line and at least one phosphorylation-independent arrestin mutant;
 - (b) separating the GPCR bound arrestin mutant from the unbound arrestin mutant; and
 - (c) determining the level of GPCR bound arrestin mutant,whereby a compound which is an agonist of said GPCR is identified when the level of GPCR bound arrestin mutant is raised relative to a control.
5. A method according to claim 4, wherein said arrestin mutant is a β -arrestin mutant.
6. A method according to claim 5, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.
7. A method according to claim 4, wherein the test compound in step (a) is furthermore brought into contact with a kinase.

8. A method according to claim 7, wherein said kinase is a G-protein coupled receptor kinase (GRK).

9. A method according to claim 1, wherein the arrestin is a labelled arrestin and the determination of the level of GPCR bound arrestin is performed by detecting signals emitted from the formed (arrestin-GPCR) complex; whereby a compound which is an agonist of said GPCR is identified when signals are emitted.

10. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises

- (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line; at least one kinase; and at least one arrestin in a suitable buffer;
- (b) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and
- (c) determining the level of GPCR bound arrestin,

whereby a compound which is an agonist of said GPCR is identified when the level of GPCR bound arrestin is raised relative to a control.

11. A method according to claim 10, wherein said kinase is a G-protein coupled receptor kinase (GRK).

12. A method according to claim 10, wherein said arrestin is β -arrestin.

13. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises

- (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line and at least one phosphorylation independent arrestin mutant in a suitable buffer;
- (b) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and
- (c) determining the level of GPCR bound arrestin,

whereby a compound which is an agonist of said GPCR is identified when the level of GPCR bound arrestin mutant is raised relative to a control.

14. A method according to 13, wherein said arrestin mutant is a β -arrestin mutant.
15. A method according to claim 14, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.
16. A method according to claim 13 wherein the test compound in step (a) is furthermore brought into contact with a kinase.
17. A method according to claim 16, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.
18. A method according to claim 10, wherein the arrestin is a labelled arrestin and the determination of the level of GPCR bound arrestin is performed by detecting signals emitted from the formed (arrestin-GPCR-carrier) complex; whereby a compound which is an agonist of said GPCR is identified when signals are emitted.
19. A method according to claim 18, wherein the signal emitted by the formed (arrestin-GPCR-carrier) complex is light due to scintillation and the arrestin is radioactively labelled.
20. A method according to claim 19, wherein the carrier material comprises scintillation proximity assay (SPA) beads.
21. A method according to claim 10, wherein the carrier material is provided with wheat germ agglutinate (WGA) to allow binding of cell membrane(s) expressing GPCR(s).
22. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises
- (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist, at least one kinase and at least one arrestin in a suitable buffer;
 - (b) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound thereto;
 - (c) separating the GPCR bound arrestin from the unbound arrestin; and
 - (d) determining the level of GPCR bound arrestin,

whereby a compound which is an antagonist of said GPCR is identified when the level of GPCR bound arrestin is lowered relative to a control.

23. A method according to claim 22, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.

24. A method according to claim 22, wherein said arrestin is β -arrestin.

25. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises

- (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist and at least one phosphorylation independent arrestin mutant in a suitable buffer;
 - (b) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace agonist previously bound thereto;
 - (c) separating the GPCR bound arrestin mutant from the unbound arrestin mutant; and
 - (d) determining the level of GPCR bound arrestin,
- whereby a compound which is an antagonist of said GPCR is identified when the level of GPCR bound arrestin is lowered relative to a control.

26. A method according to claim 25, wherein said arrestin mutant is a β -arrestin mutant.

27. A method according to claim 26, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.

28. A method according to claim 25 wherein the test compound in step (a) is furthermore brought into contact with a kinase.

29. A method according to claim 28, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.

30. A method according to claim 22, wherein the arrestin is a labelled arrestin and the determination of the level of GPCR bound arrestin is performed by detecting signals emitted

from the formed (arrestin-GPCR) complex; whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is detected.

31. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises

- 5 (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist, at least one kinase and at least one arrestin in a suitable buffer;
- (b) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound thereto;
- 10 (c) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and
- (d) determining the level of GPCR bound arrestin,
- whereby a compound which is an antagonist of said GPCR is identified when the level of
- 15 GPCR bound arrestin is lowered relative to a control.

32. A method according to claim 31, wherein the kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.

33. A method according to claim 31, wherein the arrestin is β -arrestin.

20 34. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises

- (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist and at least one phosphorylation independent arrestin mutant in a suitable buffer;
- (b) contacting at least one test compound with the resulting mixture to allow said test
- 25 compound to bind to the GPCR and thereby displace agonist previously bound thereto;
- (c) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and
- (d) determining the level of GPCR bound arrestin,
- 30 whereby a compound which is an antagonist of said GPCR is identified when the level of GPCR bound arrestin is lowered relative to a control.

35. A method according to claim 34, wherein said arrestin mutant is a β -arrestin mutant.
36. A method according to claim 35, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.
37. A method according to claim 34 wherein the test compound in step (a) is furthermore
5 brought into contact with a kinase.
38. A method according to claim 37, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.
39. A method according to claim 31, wherein the arrestin is a labelled arrestin and the
10 determination of the level of GPCR bound arrestin is performed by detecting signals emitted from the formed (arrestin-GPCR-carrier) complex; whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is detected.
40. A method according to claim 39, wherein the signal emitted by the formed (arrestin-GPCR-carrier) complex is light due to scintillation and the arrestin is radioactively labelled.
41. A method according to claim 40, wherein the carrier material comprises scintillation
15 proximity assay (SPA) beads.
42. A method according to claim 31, wherein the carrier material is provided with wheat germ agglutinate (WGA) to allow binding of cell membrane(s) expressing GPCR(s).
43. A method according to claim 1, which is a high throughput screening method.
44. Use of a compound identified according to a method as defined in any of claims 1 to 43
20 as a therapeutically effective substance.
45. Use of a compound identified according to a method as defined in any of claims 1 to 43 as a lead compound in drug design, wherein structure and/or biological properties of said compound are modified in order to provide a therapeutically effective substance.

46. A method for producing a pharmaceutical preparation comprising (i) identifying a compound using a method according to claim 1, and (ii) mixing the identified compound with a pharmaceutically acceptable carrier.
47. A method for producing a pharmaceutical preparation comprising (i) modifying the structure and/or biological properties of a compound identified using a method according to claim 1, (ii) mixing the modified compound with a pharmaceutically acceptable carrier.

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